Prediction of Stem Rust Infection Favorability, by Means of Degree-Hour Wetness Duration, for Perennial Ryegrass Seed Crops

W. F. Pfender

U.S. Department of Agriculture-Agricultural Research Service, National Forage Seed Production Research Center, Oregon State University Department of Botany and Plant Pathology, 3450 SW Campus Way, Corvallis 97331.
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ABSTRACT

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A weather-based infection model for stem rust of perennial ryegrass seed crops was developed and tested using data from inoculated bioassay plants in a field environment with monitored weather. The model describes favorability of daily weather as a proportion (0.0 to 1.0) of the maximum possible infection level set by host and inoculum. Moisture duration and temperature are combined in one factor as wet degree-hours (DH_w) (i.e., degree-hours > 2.0°C summed only over time intervals when moisture is present). Degree-hours are weighted as a function of temperature, based on observed rates of urediniospore germination. The pathogen *Puccinia graminis* subsp. *graminicola* requires favorable conditions of temperature and moisture during the night (dark period) and also at the beginning of the morning (light period), and both periods are included in the model. There is a correction factor for reduced favorability if the dark wet period is interrupted. The model is: proportion of maximum infection = $1 - e^{(-0.0031) \times (\mathrm{DHwIndex})}$, where DH_w Index is the product of interruption-adjusted overnight weighted DH_w multiplied by morning (first 2 h after sunrise) weighted DH_w. The model can be run easily with measurements from automated dataloggers that record temperature and wetness readings at frequent time intervals. In tests with three independent data sets, the model accounted for 80% of the variance in log(observed infection level) across three orders of magnitude, and the regression lines for predicted and observed values were not significantly different from log(observed) = log(predicted). A simpler version of the model using nonweighted degree hours (>2.0°C) was developed and tested. It performed nearly as well as the weighted-degree-hour model under conditions when temperatures from sunset to 2 h past sunrise were mostly between 4 and 20°C, as is the case during the growing season in the major U.S. production region for cool-season grass seed. The infection model is intended for use in combination with measured or modeled estimates of inoculum level, to derive estimates of daily infection.

Additional keywords: disease warning, infection model, *Lolium perenne*, thermal time.

Disease advisory systems increasingly have become an important tool for crop disease management. An essential component of most disease warning systems is assessment of infection favorability, usually related to weather conditions. For fungi infecting aboveground plant tissue, temperature and moisture are typically the weather parameters most closely correlated with success of infection (5,8,11). In some cases, averaged 24-h values of temperature and moisture can be used to predict infection (3); however, for other diseases, there are critical times within a diurnal cycle when temperature and moisture must be conducive for infection to occur (15,23).

Models for infection may use temperature and moisture as separate factors in the predictive equation (1,2,9,17) or may use the temperature during the moisture period as a factor, either alone (17,20) or in combination with moisture duration as a second factor (10). The infection model is commonly a sigmoid function such as the Gompertz (1), Richards (1,2,17), or Weibull (4) equation, with equation parameters expressed as polynomial functions of temperature, wetness duration, or both. In a rust disease example, de Vallevieille-Pope et al. (2) used a modified Richards equation to describe infection of wheat by *Puccinia recondita* or *P. striiformis* as a function of dew duration. The parameter for maximum infection level was a beta function of average temperature, a symmetrical curve based on minimum, optimum, and lethal maximum temperatures. Refinements may be added to such models; de Vallevieille-Pope et al. included an additional term for the adverse effect of interruption in the dew period, having observed a critical time during spore germination when interruption of moisture is detrimental to the rust pathogen germlings (2).

Although conventional time units are used generally in plant epidemic modeling, duration in models of biological phenomena may be expressed alternatively in units of thermal time such as degree-days or degree-hours. In its basic form, the concept of thermal time is that biological processes are linearly dependent on the product of time and temperature, allowing a simple calculation for prediction of development. For example, 5 h at 4°C (20 degree-hours [d-h]) would have the same effect on development as 2 h at 10°C. In addition, there is generally a low-temperature threshold below which no activity occurs, and this temperature is subtracted from the measured temperature before calculating d-h. Thermal time typically is used in phenological models for crop plants or insects, but its use in plant disease epidemiology is uncommon. Nonetheless, thermal time has been used successfully to predict inoculum production or activity by plant-pathogenic fungi (16,19,21) or bacteria populations (7).

In the Pacific Northwest, where most of the U.S. production of perennial ryegrass (*Lolium perenne*) seed occurs, an advisory system for managing stem rust (caused by *P. graminis* subsp. *graminicola*) would be beneficial to reduce costs and optimize benefits of fungicide applications. Seed crop losses due to stem rust are very severe in nonprotected perennial ryegrass stands. Fungicides are available to control the disease, and may be applied up to five times per season on susceptible seed crops. However, stem rust is

Corresponding author: W. F. Pfender; E-mail address: pfenderw@onid.orst.edu

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not equally severe in all years, and fewer fungicide applications undoubtedly are warranted in some years. Furthermore, for those applications that are made, timing could be optimized for maximum efficacy if epidemic development processes were well quantified.

Few reports have been published about infection conditions for *P. graminis* subsp. *graminicola* on grasses, with little detail concerning quantitative effects of environmental factors (13). *P. graminis* subsp. *graminis* is a different but closely related subspecies that infects wheat, and observations on the wheat pathogen are likely to be relevant to the grass pathogen. Urediniospores of *P. graminis* subsp. *graminis* germinate and form appressoria in darkness and the presence of free water, as do other cereal rust fungi such as *P. recondita*. However, the stem rust fungus is unusual among cereal rust fungi in that subsequent substomatal vesicle formation requires exposure to light while the leaves remain wet (14,18,25). Germination and appressorium formation proceed at temperatures between 15 and 29°C in the dark, with an optimum near 24°C (18), and substomatal vesicle formation is favored by temperatures of 26 to 29°C in the light. At optimum temperatures, germination and germ tube growth are completed within 4 h, appressorium formation occurs during the ensuing several hours, and some vesicles can be formed within 1.5 h of light exposure (14). The infection process thus requires specific conditions during the night (dark) period and the subsequent morning; Rowell et al. (14) found favorable infection conditions to be a dark, 7-h dew period at 24°C followed by a light period at 29°C, during which leaves dry gradually. In a limited number of field tests with morning temperatures between 13 and 23°C, Sharp et al. (18) found a good correlation between number of wheat stem rust infections and the product of average morning temperature and dew duration. They also found an effect of nighttime (dark) dew duration on the process, and suggested that temperature during the dark period could be influential.

The objective of this study was to develop an infection model based on the daily infection cycle of *P. graminis* subsp. *graminicola*. The conceptual approach is based on the assumption that there is a maximum possible level of infection, set by inoculum quantity and infection efficiency (inoculum quality interacting with host susceptibility). Further, it is proposed that environmental conditions determine the proportion of the maximum that is realized in any daily cycle of infection. The purpose of the model is to quantify this proportion based on measurable weather factors. For implementation in an epidemic prediction model, the infection model would need to be combined with other submodels, including one for estimating amount of available inoculum. In developing the infection model, a simplified approach to combining temperature and leaf wetness factors was sought, for which the use of thermal time (d-h) was evaluated.

MATERIALS AND METHODS

The basic approach to obtain data for model development was to apply a standard inoculum to potted plants, then expose them to a discrete time period (<24 h) in the field while weather conditions were monitored. Many such exposures were done, and the disease severity in each case was compared with weather measurements taken during exposure.

Field inoculum level and inoculum potential. Information was needed concerning typical amounts and infection potential of *P. graminis* subsp. *graminicola* inoculum in the air above field plantings, in order to verify that infection assay procedures were within realistic limits. All field experiments, including inoculum level measurements, were done in a 1.5-ha seed production field of perennial ryegrass cv. Morningstar, located near Corvallis, OR, and maintained with normal agronomic procedures except that no fungicides were applied to manage stem rust. A 7-day volumetric suction spore sampler (Burkhard Manufacturing Company, Rickmanworth, Hertfordshire, United Kingdom) was operated continuously from April to July each year in the perennial ryegrass stand. Air intake rate was maintained at 100 cm^3/min, and the sampler orifice was located 20 cm above the crop canopy. To count spores, the adhesive-coated tape was mounted on a glass slide, stained with aniline blue (0.075 mg/ml in 1:1:2 glycerol/lactic acid/water), and examined at ×160 magnification. Urediniospores of the pathogen were counted in sections corresponding to 2-h increments. Data were normalized to number of spores per cubic meter of air.

To measure inoculum potential (infection capacity) of the observed airborne inoculum loads, bioassay plants for inoculum detection were exposed in the field for defined time periods during spore sampler operation. The inoculum detection bioassay plants ('Morningstar' perennial ryegrass) were prepared as described previously (12), by growing to 11 weeks of age in a greenhouse with supplemental light to provide 14-h day length. Each plant was in a separate, cylindrical pot ("cone-tainer") of vermiculite. Ten plants were used for each exposure. The leaf area was approximately 0.1 m^2 per 10 plants, as estimated by destructive sampling of additional plants at the time of exposure and scanning leaves with a leaf area meter (Area Meter A100; ADC Plant Science Instrumentation, Hoddesdon, Hertfordshire, England). During exposure, plants were set in the field within 0.5 m of the spore sampler orifice, with their top leaves at the height of the crop canopy, between 0800 and 1700 h. They then were returned to the laboratory and incubated under conditions highly favorable for infection (12); that is, misted in the dark at 18°C for 8 h followed by 4 h in the light at 22°C as the leaves gradually dried (14). As a check for extraneous infection, 10 plants that were not exposed in the field were included in the overnight incubation. After this treatment, plants were returned to a greenhouse where they were maintained for 2 weeks at 22 to 26°C. During this time, moisture in the pots was maintained by standing them in pans of water, so that the leaves were never wet. This procedure prevented any infections from occurring subsequent to field exposure. Two weeks after exposure in the field, the total number of uredinia on the 10 plants was counted. The test was conducted 11 times in each of the years 1998 and 1999. The sum of pustules on the 10 exposed plants in each test was used as a single observation in the regression analysis of infection versus airborne spore load during the hours of plant exposure.

Infection bioassay. Potted plants were grown to 11 weeks of age, as described. Inoculum for each year was a bulked collection of urediniospores obtained the previous summer from several perennial ryegrass cultivars and locations, then dried and stored at –60°C and heat shocked before use (12). Spores were suspended in light mineral oil (12 mg of spores per milliliter of oil, approximately 5×10^6 spores per ml) and sprayed onto plants at a rate of 1.0 ml per 50 plants. The leaf area exposed to inoculum was approximately 0.1 m^2 per 10-plant replicate. The inoculum density was approximately 3×10^2 spores per cm^2, as determined by counting spores deposited on glass slides placed among the grass leaves before spraying. After allowing 1 h for oil to evaporate from plants, 10 of the plants were placed in a dew chamber under conditions favorable for infection (12) as a check on inoculum viability and to measure a maximum level of infection from the applied inoculum under ideal infection conditions. The other 40 plants were transported to a 1.5-ha field of perennial ryegrass at the Hyslop experiment farm near Corvallis, OR. At approximately 1700 h on each trial date, 10 plants were placed in each of four randomly selected locations in the field, with their leaves at the level of the top of the crop canopy. At noon the following day, all plants were returned to a greenhouse, where they were maintained at 22 to 26°C with no leaf wetness, as described previously. Two weeks after exposure to field conditions, the number of uredinia on each 10-plant set was counted. Data from the four sets were averaged to produce one estimate of infection level for each trial

date. Exposure trials were conducted approximately every 2 weeks during March through July 1998, 1999, and 2000 and September through October 1999. On eight dates in March through June 2001, the test procedure was modified to include three levels of applied inoculum. In these tests, 50 plants were inoculated as described previously, an additional 50 plants received one-fifth as much inoculum (1×10^6 spores per ml), and an additional 50 plants received 1/25 as much (2×10^5 spores per ml).

In summer of 2001, several inoculations of field-grown plants were conducted. In these cases, inoculum was prepared as described above and applied to plants in a stand of 'Morningstar' perennial ryegrass sown the previous fall. Inoculations were performed in the afternoons of 17 and 26 April, 16, 23, and 28 May, and 6 June. As a check treatment, some plants received no inoculum. Weather data were recorded during the following night and morning. Each of these trials was scored approximately 1.2 latent period durations (12) after the inoculation date, and the severity of stem rust was expressed as number of pustules per 0.1 m^2 of leaf area present at the time of inoculation.

A possible source of error in these infection bioassays is variation in background levels of airborne inoculum in the field, which could inflate disease severity beyond that from the applied standard inoculum. Measurement of airborne inoculum obtained from the Burkhard spore sampler during each exposure period of infection bioassay plants was used to adjust for this possible artifact. The number of infections expected due to the measured airborne inoculum was calculated, using results from the inoculum potential assays described previously, and the ratio of applied inoculum potential to total inoculum potential (applied plus background) for each trial was determined. The measured disease severity in the infection bioassay was multiplied by this ratio to obtain the level of infection attributed to the applied standard inoculum, and this adjusted disease level was used as the data point for that trial. In 75% of the trials, the background inoculum level was less than 5% of the applied inoculum, and in no case was the background inoculum level greater than twice the applied inoculum.

Weather data. Weather conditions at the field site were monitored with an electronic datalogger (CR10X; Campbell Scientific Inc. [CSI], Logan, UT). Air temperature at canopy height was measured with thermistor probes in standard shields (107-L temperature probe, 41301 shield; CSI). Leaf wetness duration was estimated with flat-plate resistance sensors (LWS 237; CSI) mounted at a 45-degree angle facing west, painted with pale green latex paint (22), and maintained approximately 5 cm below the top of the canopy. Relative humidity (probe HMP35C; CSI), windspeed (03001 Wind Sentry; CSI), solar flux (L1200X pyranometer; CSI), and precipitation (tipping bucket gauge TE525; CSI) were measured at 1.5 m above the ground, approximately 1 m above canopy height, at one location in the field. Temperature probes and LWS were located at each of four sites within the field where test plants were placed.

Weather factors were recorded every 30 min. Whereas the windspeed and LWS records were single-time readings at this interval, the 30-min records for temperature, relative humidity, and solar flux were averages of six readings taken at 5-min intervals. The precipitation records were sums for each 30-min interval. Leaf wetness duration was calculated from the weather data as a binary (+ or –) variable for each time interval. An interval was considered wet if any of the following was true: LWS resistance < 4,500, precipitation > 0, or relative humidity > 95%. Sunset and sunrise times were calculated (from date, latitude, and longitude) as a subroutine in the data collection program, and were within ±3 min of actual sunset and sunrise times.

Relationship of infection severity to process rates. A model relating number of infections to weather conditions can be constructed through the use of rates. Of all the spores arriving at the plant surface and capable of successfully infecting the host during an event of prolonged conducive conditions, not all will complete the process in the same amount of time. Some will proceed more slowly than others so that, at progressively shorter durations of conducive conditions, progressively fewer spores will achieve infection before conditions become unsuitable. Also, if the average rate of completion is proportional to temperature then, for a given time duration, the number of successfully completed infections will be proportional to temperature. The effects of temperature and duration can be combined as d-h. Under the above assumptions, it is expected that the number of successful infections would increase with increasing d-h of conducive conditions, up to an asymptotic maximum representing the maximum infection efficiency. As noted previously, strict application of the d-h concept assumes linearity across temperatures. However, there will be deviations from this linearity if incremental heat near the low-temperature threshold or lethal upper temperature is relatively ineffective, or if there is a maximum rate above which higher temperatures cannot induce proportional increases. The deviations from linearity can be expressed as the relative effectiveness of d-h compared across different temperatures.

Urediniospore germination rate as a function of heat units. To develop a thermal-time model for infection, information was needed about lower and upper threshold temperatures for infection, and nonlinearity of d-h effects. Spore germination is the necessary initial step of the infection process, and is easy to measure across a range of controlled conditions. Although germination does not represent all components of the infection process, which may differ in their absolute response to temperature, a reasonable starting point for model development is to consider that temperature response of germination reflects the *relative* effectiveness of d-h across different temperatures.

In vitro experiments were conducted in which urediniospores were exposed to defined, constant temperatures and germination was quantified over time. Freshly collected urediniospores of *P. graminis* subsp. *graminicola* were transferred to the surface of water agar plates by means of a dry brush, at a density of approximately 200 spores per cm^2. This density is safely below 1,600 spores per cm^2, the level above which significant germination inhibition due to self-inhibitors has been observed (24). The plates, which had been equilibrated at the desired temperatures for at least 1 h before the transfer, were returned immediately to their respective incubators and maintained in the dark for specified lengths of time. At the designated time, each plate was removed from the incubator and a drop of acetone was applied to the spores to prevent further germination. The plates were examined at ×160, and 100 spores per replicate plate were observed for germination, defined as the presence of a germ tube longer than the spore diameter. There were two replicate plates per temperature per time interval. Target temperature treatments were 2, 4, 6, 10, 16, 22, 28, 30, and 32°C. Actual temperatures were measured by mercury-in-glass thermometers kept in each incubator and used in the analyses. The experiment was performed twice.

Model development and testing. Scatter-plot graphs were made and linear correlation coefficients calculated for the 1998 and 1999 March-to-July infection assay data set (1998–1999 data), with disease severity (log [number of pustules + 1] per 10 plants) as the dependent variable plotted against each of several independent variables. The independent variables were nighttime values for average or minimum air temperature; average relative humidity, precipitation, or hours of leaf wetness; morning (the first 2, 2.5, 3, 3.5, or 4 h after sunrise) values for average temperature; average relative humidity; precipitation; solar flux; or hours of leaf wetness. Independent variables correlating highly with disease were selected, and outliers from these correlations were examined individually to select other variables that could explain important deviations. Various transformations of moisture and temperature, including cumulative d-h, were assessed as predictive measures of observed infection severity. Interruption in

wetness duration, reported in other research to influence rust spore germination (2,6), was tested as an additional descriptive variable. Nonlinear, least-squares curve fitting with the Marquardt-Levenberg algorithm was done for several sigmoid curves in SigmaPlot software (SPSS, Inc. Chicago), and the best model (based on coefficients of determination, residuals pattern, and the predictive residual error sums of squares) was selected. In order for the model to produce a prediction of relative infection favorability (between 0 and 1.00), the dependent variable was expressed as a proportion of the maximum infection level (obtained under controlled conditions); therefore, the model parameter for maximum level was set to 1.00, and the remaining parameters were estimated for this 1998–1999 data set.

To validate the performance of the model derived from 1998–1999 data, the observed infection values for the 2000 infection tests (which included autumn 1999 and summer 2000 trials) were compared with values predicted by the 1998–1999 model applied to the 2000 weather data. Likewise, observed infection levels in the 2001 multiple-inoculum level tests were compared with predicted values, as were results from the 2001 inoculations of field-grown plants. All validation tests were analyzed by linear regression of observed versus predicted values.

RESULTS

Patterns of airborne inoculum and correlated infection potential. There was a clear diurnal pattern for urediniospore levels in the air above the grass seed crop canopy (Fig. 1A). Peak spore concentration occurred between 1000 and 1600 h. Between 0600 and 1700 h (i.e., roughly the daylight hours before 1700 h), 72% (in 1998) or 80% (in 1999) of the total daily spore load was present. Total daily inoculum present during the interval varied greatly among days (Fig. 1B), as would be expected with change in level of disease in the canopy during the season. Daily cumulative airborne spore loads were as high as $10^{5.5}$ urediniospores per m^3 during the 0600-to-1700-h time period. Number of infections on plants exposed to natural inoculum was proportional to airborne spore loads (Fig. 2). Above the lower limit of detection by the spore sampler (10 spores per m^3 accumulated over 10 h), the amount of disease resulting under optimum infection conditions was estimated as log (pustules per 0.1 m^2 of leaf area) = 0.82 (log [spores per m^3 of air]) – 0.86. Thus, for example, infection levels of 10^3 pustules per 0.1 m^2 of leaf area occur under optimum infection conditions after exposure to airborne inoculum loads of approximately $10^{4.7}$ spores per m^3. Levels of airborne inoculum this high or higher occurred on 11% of the days in 1998 and 14% of the days in 1999 (Fig. 1B). From these observations it was determined that, to conduct bioasssays for the infection model, an abundant but realistic daily inoculum level can be represented by applying an inoculum capable of producing 10^3 pustules per 10 plants, and placing the plants in the field at 1700 h.

Spore germination response to temperature and thermal units. Urediniospores of *P. graminis* subsp. *graminicola* germinated rapidly between 11 and 28°C, with a rate maximum near 23°C, and there was no germination at 2 or 32°C (Table 1; Fig. 3A). In order to use temperature as a predictor for stem rust infection, a function relating temperature to fungus activity is needed. The spore germination data (Table 1) were analyzed to evaluate the use of d-h to summarize the effect of temperature on activity. For convenience in the comparison of temperature effects, the duration required to reach 10% germination was selected as the response for analysis, because this is approximately the level that was achieved at the coldest tested permissive temperature after 10 h (Table 1), a typical nighttime period. The number of hours required to reach 10% germination was estimated by interpolation within the time interval that included 10%. Germination rate (reciprocal of time to reach 10% germination) shows a typical temperature response (Fig. 3A), which can be described well with

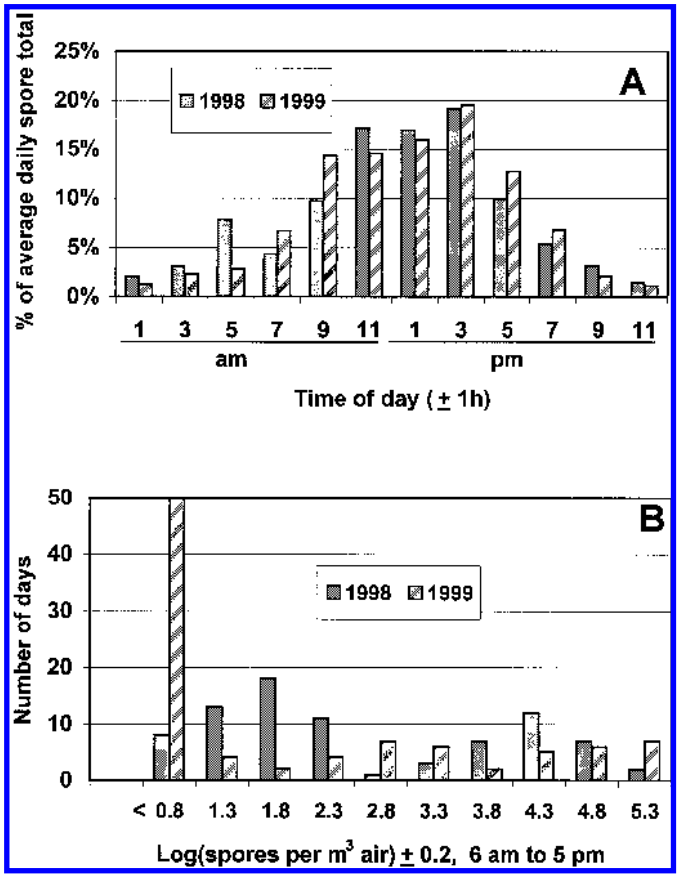

Fig. 1. Urediniospore density in the air above a perennial ryegrass seed crop infected with *Puccinia graminis* subsp. *graminicola*. **A,** Bihourly frequency distribution of spore density, determined for 2-h intervals continuously in April, May, and June 1998 and 1999. Height of bars represents percent of the average daily spore density occurring in each 2-h interval, averaged within each year. **B,** Frequency distribution for daytime urediniospore densities during April, May, and June 1998 and 1999. Height of bars represents number of days during this period in each year when the cumulative spore density collected between 0600 and 1700 h was within the indicated range.

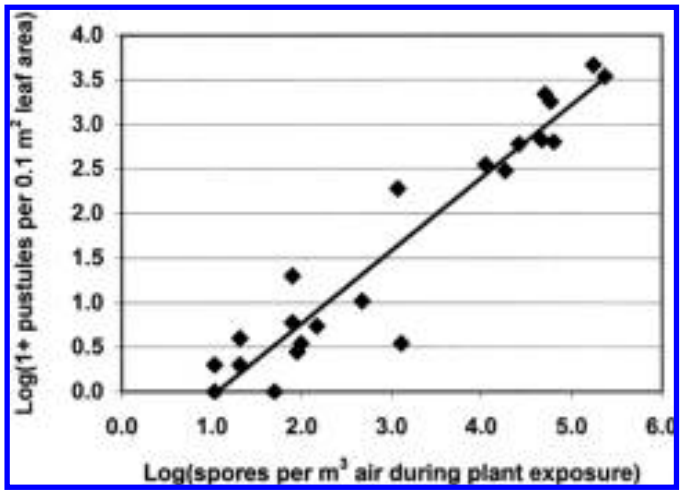

Fig. 2. Infection potential of *Puccinia graminis* subsp. *graminicola* urediniospores deposited onto plants from the air over a field of infected perennial ryegrass. Bioassay plants were exposed for 10 h in a field while spore densities were measured with a Burkard spore sampler. Plants then were incubated under optimum infection conditions in the absence of additional inoculum. Sampler detection limit for urediniospores per cubic meter of air over the 10-h period was 10 spores. Linear regression equation is: log (pustules) = 0.82 (log [spore density]) – 0.86, adjusted $r^2 = 0.91$.

a fourth-degree polynomial. To calculate d-h required to reach 10% germination, duration was multiplied by effective temperature, defined as ambient temperature minus the low-threshold value of 2°C, chosen because this was the lower limit observed for germination (Table 1). If the response to temperature were strictly linear, the number of d-h required for a given response (e.g., 10% germination) would be the same at all temperatures. We observed that these values are roughly similar across a range of temperatures (14.2 ± 0.9 d-h from 6 to 23°C), but deviate more markedly at higher and lower temperatures. Rates, calculated as percent germination per d-h, were derived as the reciprocal of number of d-h required to reach 10% (multiplied by 10 to obtain units of percent germination). Then, a weighting of heat-unit effects for different temperatures was calculated simply as the relative magnitude of the d-h rates at different temperatures. Comparison of these relative germination rates (Table 1) shows that the highest germination rate (percent per d-h) occurred at 11°C, and that the rate per d-h at 28°C was only approximately half as great as the maximum. The data from the experiment in Table 1 were combined with data from the repeated experiment, and a polynomial was fit to the data to create a weighting function for relative d-h rates for all temperatures between the minimum and maximum for germination (Fig. 3B). This equation, expressing d-h rate as a function of temperature, combines temperature effects on hourly rate (Fig. 3A) with basic and nonlinearity features of thermal time rate. The relative d-h rate for any temperature, as calculated from the polynomial function, can be used as a weighting factor for the effect of d-h at that temperature. Thus, for example, 10 d-h occurring at 15°C (which requires 43 min) will result in the same amount of pathogen development as 10 d-h at 4°C (requiring 300 min), but only approximately 80% as much development as 10 d-h at 8°C (requiring 100 min).

Development of infection model. Correlation between weather variables and infection severity was examined. Although spurious correlations may be found when a large number of factors is tested for correlation, this part of the analysis primarily was intended to suggest ideas for further exploration and modeling. For the 1998–1999 data, the weather variables explaining the highest proportion of infection events were the average and low night temperatures and average morning temperature (Table 2; Fig. 4A and B). Inspection of regression coefficients of determination for values of weather variables measured during the first 1.0, 1.5, 2.0, 2.5, 3.0, and 3.5 h after sunrise showed that values for the first 2.0 h were equally or more highly correlated with infection than values for shorter or longer periods following sunrise; Figure 4B and D illustrates conditions during the first 2 h after sunrise. Morning solar intensity (Table 2) was inversely correlated with hours of morning leaf wetness (slope coefficient = –1.25, adjusted r^2 = 0.60), presumably reflecting more rapid leaf drying on sunny mornings. The nighttime or morning leaf wetness durations were not, by themselves, highly correlated with infection level (Fig. 4C and D), but examination of individual data points in the infection versus temperature plots (Fig. 4A and B) showed that most of the outliers were cases of unusually long or short wetness duration.

From these correlation observations, a model was developed based on the known biology of *P. graminis* (14,18) and results of the experiments on germination across temperatures. The model is based on temperature and wetness, with the following rationale: the fungal processes necessary to establish infection can proceed only in the presence of moisture, and the time required for establishment can be modeled as thermal time units (weighted for nonlinearity across temperatures) (Fig. 3B) during moisture periods. The predictive variable is thus the temperature-weighted d-h of wetness, designated here wet degree-hours (DH_w). The sum of overnight DH_w is a reasonably good predictor of infection (Fig. 4E). In linear correlations of infection level with morning DH_w summed over various time periods after sunrise, the r^2 values for durations of 1.0, 1.5, 2.0, 2.5, 3.0, and 3.5 h after sunrise were 0.79, 0.79, 0.78, 0.69, 0.60, and 0.53, respectively; Figure 4F illustrates the data for the first 2 h after sunrise. The separate infection processes which occur in the dark (spore germination and appressorium formation) and after sunrise (substomatal vesicle formation) are both required for successful infection (18); therefore, it is reasonable to expect that infection likelihood would be a product of overnight and morning favorability, and Figure 4G illustrates correlation of this product (nighttime DH_w × post-sunrise DH_w, designated "DH_w Product") with observed infection level. The relationship between DH_w Product and infection level (Fig. 4G) appears to be in the form of an exponential saturation equation (i.e., infection level = $k[1 - e^{bx}]$), where k = the maximum, b = a parameter, and x = DH_w Product. Several calculations of the DH_w Product, differing in the post-sunrise duration used to calculate morning DH_w, were fitted to this equation using SigmaPlot software (SPSS, Inc.). The adjusted r^2 values for infection versus the DH_w Products calculated with post-sunrise durations of 1.0, 1.5, 2.0, 2.5, 3.0, or 3.5 h were 0.76, 0.79, 0.82, 0.78, 0.74, and 0.72, respectively. From these analyses, it was concluded that the best form of the DH_w product for predicting infection level is the morning DH_w for the first 2 h after sunrise multiplied by the nighttime DH_w.

It was observed that several trials having substantially less infection than predicted from the DH_w Product had an interrupted nighttime wetness period. For this reason, as well as other reports of decreased rust spore germination with interruption of the wet period (2,6), a wetness interruption factor was estimated. For the estimation, the beginning and end of the sensitive time period (in d-h, starting at sunset) and magnitude of the factor were iteratively adjusted to minimize the squared difference of observed minus

TABLE 1. *Puccinia graminis* subsp. *graminicola* urediniospore germination response to temperature (T), and derivation of degree-hour (d-h) germination rates and proportional factors for temperature

	Germination (%) at indicated incubation durations (h)							Duration[a]		Germination rate	
T (°C)	0.5	1.0	2.0	34.0	4.0	8.0	10.0	Hours	d-h[b]	Percent per d-h[c]	Relative[d]
2.0	…	0	…	…	0	…	0	…	…	0	0
3.8	…	0	…	…	2 + 1	7 + 2	10 + 1	10.00	18.0	0.556	0.69
6.2	…	0	0	9 + 1	36 + 5	42 + 2	…	3.04	12.8	0.783	0.97
11.0	…	0	27 + 2	…	…	…	…	1.37	12.3	0.811	1.00
16.8	0	2 + 1	92 + 1	…	…	…	…	1.09	16.1	0.620	0.76
22.6	0	22 + 10	94 + 2	…	…	…	…	0.73	15.0	0.665	0.82
27.6	0	12 + 1	…	…	…	…	…	0.92	23.6	0.425	0.52
29.7	…	0	25 + 5	…	…	…	…	1.40	38.8	0.258	0.32
32.0	…	0	…	…	0	…	0	…	…	0	0

[a] Duration to reach 10% germination.
[b] Hours × (temperature – low threshold), with low threshold = 2°C.
[c] Rate is percent germination per d-h, obtained as 10 × (the reciprocal of d-h to reach 10% germination).
[d] Maximum = 1; d-h rate of germination expressed as a proportion of the maximum rate observed, to provide a weighting factor for nonlinearity of d-h rate with temperature.

predicted values for the fitted exponential saturation equation. The result was a factor = 0.64, to be multiplied by the DH_w Product if there is an interruption in the nighttime wet period after 5 DH_w have accumulated but before 15 DH_w have elapsed. The improvement in prediction due to use of the interruption factor was minor overall (adjusted r^2 improved from 0.82 to 0.84), but overprediction was ameliorated. Comparison of Figure 4G (no interruption factor) with Figure 4H (interruption factor applied) shows several cases in which the d-h predictor value is decreased (moved to the left), and closer to the model line. A one-third and a one-half reduction in spore germination due to interrupted wet period have been reported previously for bean rust (6) and wheat leaf rust (2), respectively. The interruption-adjusted DH_w Product, designated the DH_w Index, is shown in Figure 4H as the predictor variable for stem rust infection level expressed as a proportion (0 – 1.0) of the maximum. In the 1998 and 1999 infection bioassay tests, the maximum infection levels ($\log_{10}$[maximum]) and standard deviations (SD) obtained under optimum conditions were 2.99 (SD = 0.19) and 3.06 (SD = 0.36), respectively. The fitted equation is: Proportion of maximum infection = $1 - e^{(-0.0031) \times (\text{DHwIndex})}$, with an adjusted r^2 of 0.84. The estimate of the exponential parameter (0.0031) had a standard error of 0.0008 and a P value of 0.0003. This is the proposed model for favorability of weather for infection of perennial ryegrass by *P. graminis* subsp. *graminicola*.

Model validation. The model was tested with three different data sets, each independent of the others and of the data used for model development. Within each validation data set, the model equation was used to predict the proportion of maximum possible infection that would occur under the weather conditions of each overnight trial. These maxima could differ among years, because of differences in quality of the inoculum (collected the previous year for each set of validation trials). The maximum possible infection level (pustules per 0.1 m^2) for each data set was determined by averaging the maximum infection levels obtained under optimum controlled conditions using the inoculum for that set of trials. The values, expressed as ($\log_{10}$[maximum]) and their SD were: 2000 test, 3.20 (SD = 0.10); 2001 inoculum level test, full inoculum level 3.38 (SD = 0.29), one-fifth inoculum level 2.84 (SD = 0.26), 1/25 inoculum level 2.14 (SD = 0.18). Validation trials with field-grown plants in 2001 used the same inoculum as the 2001 full-inoculum level (3.38, SD = 0.29).

For the trials in the 2000 data set (Fig. 5A), the modeled values accounted for 84% of the variation in observed values. The least-squares linear regression line for observed versus modeled values had a slope not significantly different (5% level) from 1.0 and intercept not significantly different from 0.0. Likewise, in the 2001 validation test using three different inoculum levels on each of the trial dates (Fig. 5B), the regression line for observed versus modeled values was not significantly different in slope or intercept from observed = modeled, and the adjusted r^2 was 0.80. Results of the six trials in the validation set conducted by in-field inoculation of field-grown plants (Fig. 5C) also plot close to the line modeled = observed.

Implementation of the infection model could be simplified computationally if one could assume that the infection response to thermal time units is independent of temperature. If the relationship shown in Figure 3B can be approximated by a straight horizontal line between 2°C and an upper limit (≈30°C), one could use simple d-h (without weighting) for calculating the index, here designated "simple DH_w Index". To examine this possibility, thermal time units in all trials were recalculated as (duration × [temperature (°C) – 2.0]), with no weighting, and a new model was constructed and tested from the same raw data sets used to construct and test the weighted model already described. The nonweighted model developed from the 1998–1999 data was: Proportion of maximum infection = $1 - e^{(-0.0028) \times (\text{simple DHwIndex})}$, with an adjusted r^2 of 0.83 and P value of 0.0002 for the exponential parameter. When this model was used with nonweighted d-h to predict infection levels in the 2000 and 2001 validation data sets, results were similar to those obtained with the weighted d-h model. The regression coefficient of determination (adjusted r^2) for observed versus modeled values was 0.84 for the 2000 data and 0.79 for the 2001 inoculum-level validation test; for both validation data sets, regression intercepts and slopes were not significantly different (5% level) from 0.0 and 1.0, respectively. For the 2001 validation trials with field-grown plants, the modeled results were close to the observed results, as seen for the weighted d-h model. A comparison of the simple and weighted d-h models is illustrated in Figure 6A and B. In each case, data points from all

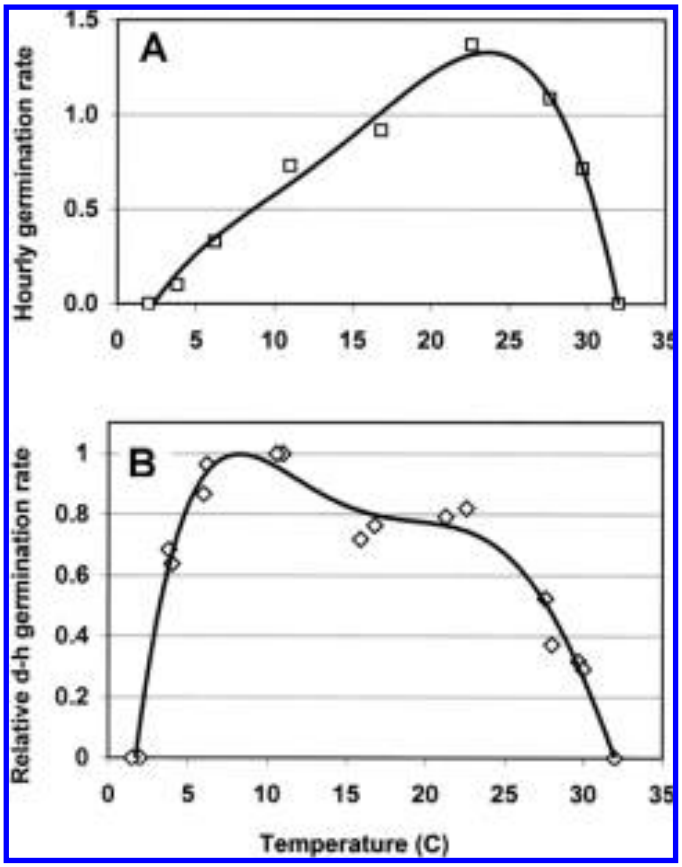

Fig. 3. Germination rate of *Puccinia graminis* subsp. *graminicola* urediniospores, tested on water agar at constant temperatures. Table 1 provides derivation of rates. **A,** Germination rate expressed as percent germination per hour. Fitted curve is a fourth-order polynomial of temperature. **B,** Germination rate expressed as percent germination per degree-hour, then normalized to maximum rate of 1.0. Polynomial function is: Relative rate = $(1.064 \times 10^{-6})T^5 - (1.145 \times 10^{-4})T^4 + (4.50 \times 10^{-3})T^3 - (8.126 \times 10^{-2})T^2 + 0.654T - 0.905$, where T = temperature (°C) between 2 and 32.

TABLE 2. Regression analysis for weather variables as predictors of observed bioassay infection levels, perennial ryegrass stem rust 1998–1999 tests

Weather variable[a]	Slope coefficient	Coefficient of determination (adj. r^2)
Morning average temperature (°C)	0.239	0.46
Morning cumulative solar flux (MJ/m^2)	–1.410	0.30
Morning average relative humidity	0.051	0.25
Morning leaf wetness duration (h)	0.686	0.20
Night average temperature (°C)	0.201	0.44
Night minimum temperature (°C)	0.204	0.46
Night average relative humidity	0.047	0.23
Night rain (mm)	–0.016	0.09
Night leaf wetness duration (h)	0.060	0.04

[a] Morning = first 2 h after sunrise. Night = sunset to sunrise.

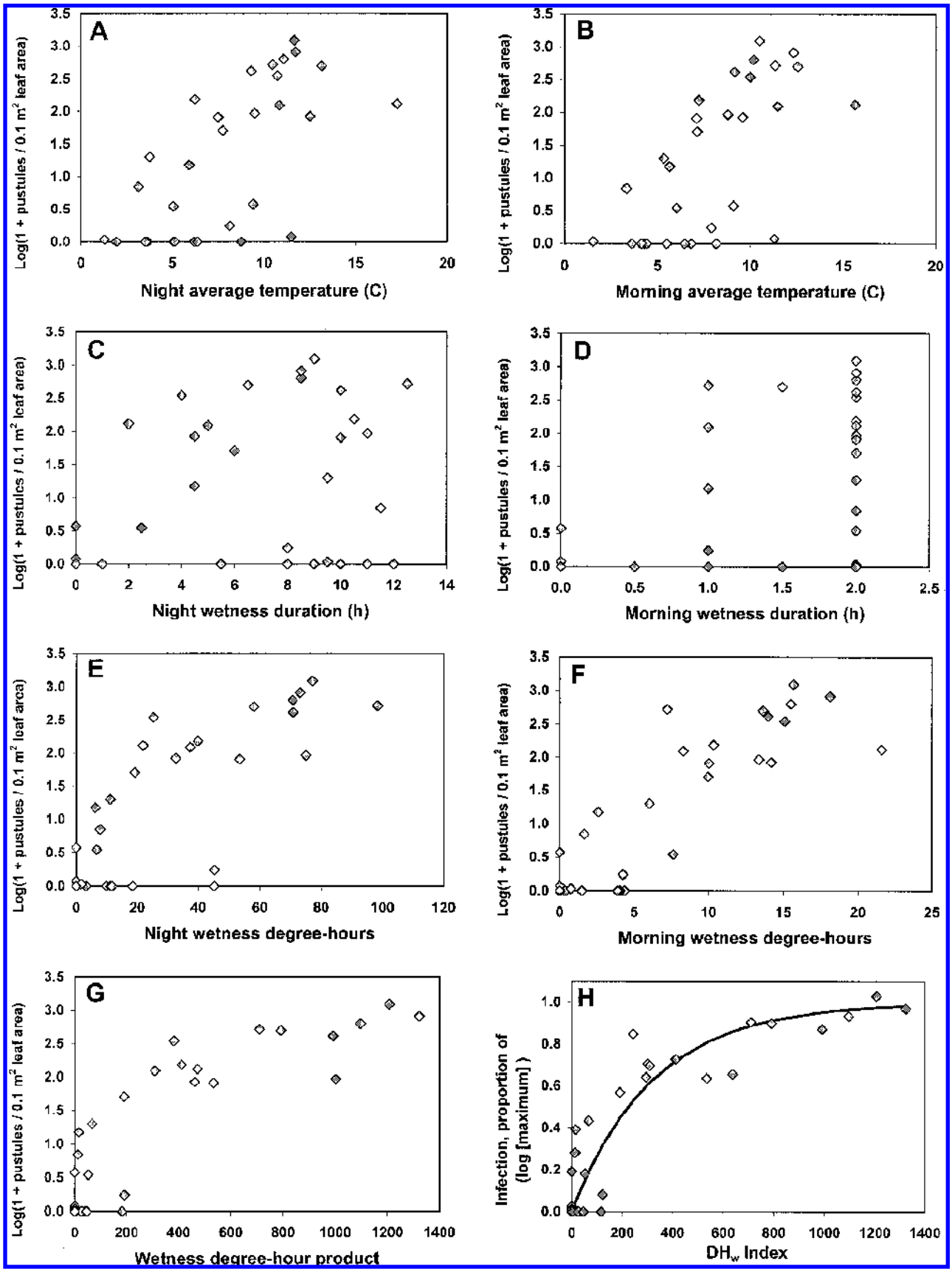

Fig. 4. Relationship of infection level to selected temperature and moisture variables, in data from April to July 1998–1999. Each data point represents one trial of 40 plants. Predictor variables are: **A,** average temperature during dark (nighttime) hours, **B,** average temperature during the first 2 h after sunrise, **C,** hours of leaf wetness during dark hours, **D,** hours of leaf wetness during first 2 h after sunrise, **E,** degree-hours (low threshold = 2.0°C) of leaf wetness during dark hours, **F,** degree-hours of leaf wetness during first 2 h after sunrise, and **G,** product of (dark degree-hours of wetness) × (morning degree-hours of wetness). **H,** Model for prediction of stem rust infection: Infection (proportion of maximum) = $1 - e^{(-0.0031) \times (\mathrm{DHwIndex})}$, where DH_w Index is the product of overnight and morning degree-hours of wetness, multiplied by 0.64 if the dark wetness period is interrupted.

three validation sets (2000, 2001 inoculum level tests, and 2001 field-grown plants test) are plotted together. Overall performance is similar between the two models, as is evident by the similar coefficients of determination (adjusted $r^2 = 0.80$ for both), slopes, and intercepts. The two models give very similar predictions under nonpermissive (no infections) or moderately to highly favorable (observed values > 2.0) conditions, but the simplified model slightly overestimates many of the marginally favorable infection events (observed $\log_{10}$[infections] between 0.5 and 1.5).

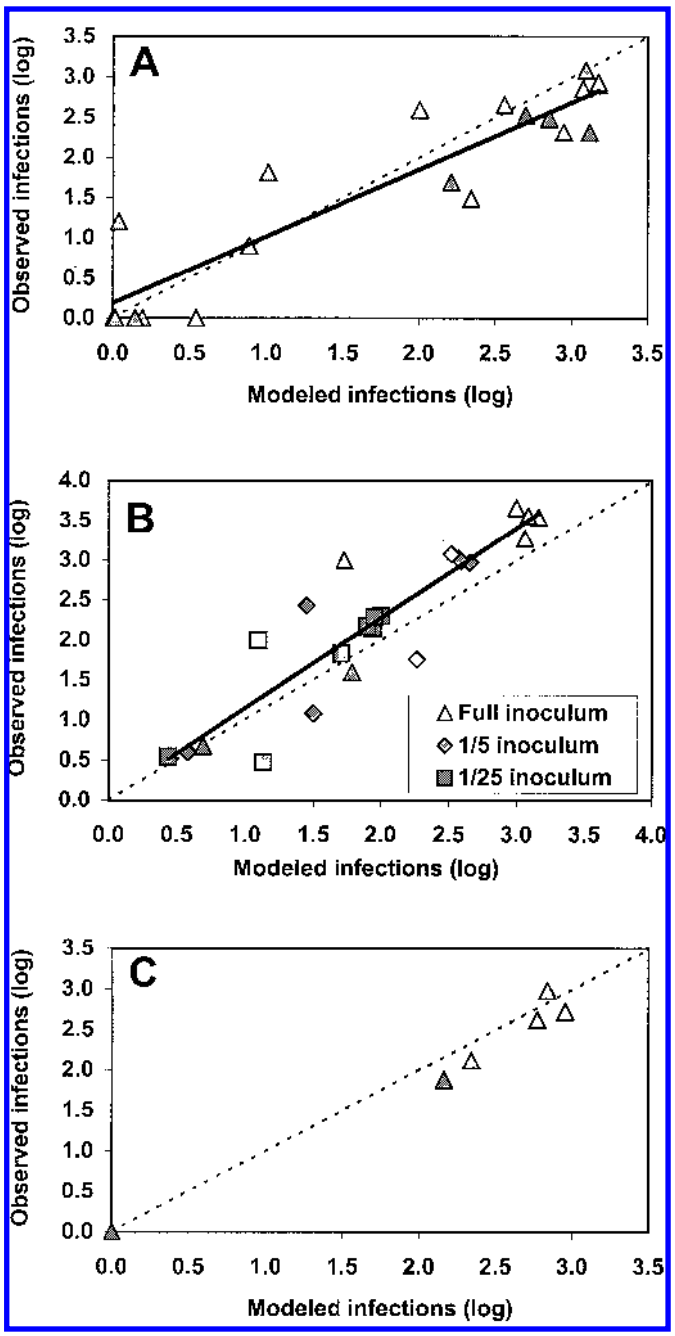

Fig. 5. Validation tests for stem rust infection model on perennial ryegrass. The model shown in Figure 4H was applied to weather data collected during individual infection trials conducted during 2000 and 2001. Infection predicted by the model is compared with observed infection. Each data point represents one trial of 40 plants. **A,** Modeled and observed infection levels for trials conducted in autumn 1999 and summer 2000. The regression line (solid line) is not significantly different from observed = modeled (dashed line), and the coefficient of determination (adjusted r^2) = 0.84. **B,** Modeled and observed infection levels for trials conducted in 2001, in which three different inoculum levels (full, one-fifth, and 1/25) were used in each trial. The regression line (solid line) is not significantly different from observed = modeled (dashed line), and the coefficient of determination (adjusted r^2) = 0.81. **C,** Modeled and observed infection levels in trials using in-field inoculation of field-grown plants in 2001. The dashed line represents observed = modeled.

DISCUSSION

The goal of this research was to develop a model to quantify the effects of weather on infection of perennial ryegrass by *P. graminis* subsp. *graminicola*. The underlying operational concept is that there is a maximum possible infection level for any specific combination of host and inoculum, and that measurable weather factors determine the proportion of this maximum possible infection that is realized. The model is intended to quantify this proportion.

Several assumptions were made in designing the bioassays and developing the model. First, infection is assumed to be affected by conditions during two distinct phases, the night (dark) and the early morning periods, with both being necessary and quantitatively influential for infection. Further, rate of infection is controlled by temperature, provided that moisture is present. This view of the infection biology for *P. graminis* is supported by previous research (14,18,25). A related assumption is that the relative effect of temperature on spore germination rate can be used as a surrogate for the relative effect of temperature on rate of the entire infection process from germination to substomatal establishment. Although the temperature response of post-germination infection processes may differ from that of spore germination, it seems reasonable to suppose that the relative response of all pro-

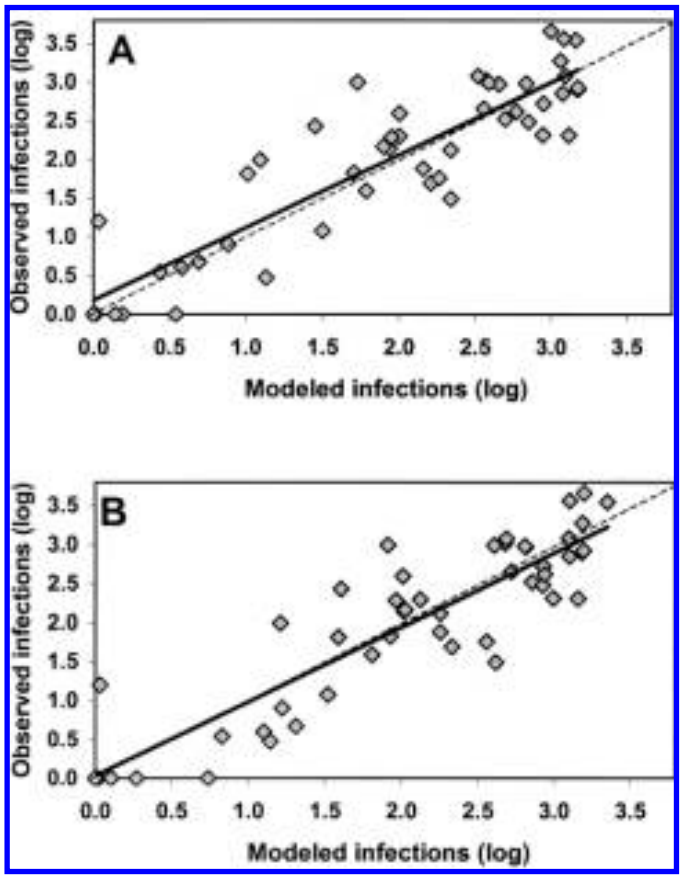

Fig. 6. Comparison of weighted and nonweighted degree-hour models. Combined data for all validation tests (2000 and 2001). The regression lines (solid) and lines for observed = modeled (dashed) are shown. **A,** Observed infection levels versus infection levels modeled using the weighted degree-hour index. The regression line (adjusted $r^2 = 0.79$) is not significantly different from observed = modeled. **B,** Observed infection levels versus infection levels modeled using the nonweighted degree-hour index. The regression line (adjusted $r^2 = 0.80$) is not significantly different from observed = modeled.

cesses to a range of temperatures will be sufficiently similar that they can be estimated from the relative effects on germination response. A further aspect of this assumption is that the low-temperature threshold for infection is represented by the low-temperature threshold for germination. It is possible that other components of the infection process (e.g., appressorium formation) may have a higher temperature threshold. However, the infection model based on this threshold was adequate to predict observed infection levels at low temperatures. Also, it is noteworthy that infection was observed in these experiments, in some cases, where measured temperatures during the dark and early morning periods ranged from 2.8 to 5.0°C (1998–1999 data, Fig. 4A and B). These apparent minimum temperatures for successful infection of perennial ryegrass by *P. graminis s*ubsp. *graminicola* are substantially lower than the 15°C reportedly required by *P. graminis* subsp. *graminis* infection of wheat (18). We previously found that latent-period duration for stem rust on perennial ryegrass is well modeled as a d-h dependent process with a low threshold of 1.5°C (12).

Another assumption is that germination rate (and, by generalization, rate of infection processes) can be predicted by a d-h calculation adjusted for nonlinearity across temperatures. Thermal time units (even without adjustment for nonlinearity) have been used to describe pathogen phenology in other studies (7,16,19,21), and the approach appears to work well here for the infection model. Note that using a temperature function for weighting d-h rates (Fig. 3) is computationally equivalent to developing a temperature function for conventional rates (germination per hour) and multiplying these by duration. The advantage of d-h (thermal time units) is in the potential for simplified calculation of temperature effects (i.e., where development is proportional to temperature × duration across all temperatures. When the model was paramaterized and tested with a simple d-h calculation (not weighted for nonlinear temperature effects) (Fig. 6B), the nonweighted d-h calculation was nearly as good as the weighted form in predicting infection, at least for stem rust of perennial ryegrass in this climate. The probable reason that the simplified approach is adequate is that most overnight and morning temperatures are within the range of 4 to 20°C, as illustrated by a weekly sampling of daily temperature ranges (sunset to 2 h after sunrise) during March through July in 1998 and 1999 (Fig. 7). In this range (4 to 20°C), the d-h rates are relatively independent of temperature, differing by only ±5% (Fig. 3B). The most marked differences between the weighted and nonweighted d-h models in predicting infection levels (Fig. 6A and B) occur in low-probability (but > 0) infection events. These are usually low-temperature events or short-moisture events, the latter typically associated with high temperatures. The d-h rates deviate downward substantially only at temperatures below 4 or above 20°C (Fig. 3B); therefore, the simple d-h model would be likely to overestimate only the low-probability infection events that result from especially cold or warm temperatures. In climates where sustained overnight and early-morning temperatures below 4°C or above 20°C are common, the weighted model would be needed to accurately predict these low-probability events. In deriving the weighting for the d-h effects of temperature, a fifth-order polynomial was used (Fig. 3B). A simpler curve also was tried, but with less satisfactory results. When the model was recalculated using d-h weightings from a third-order polynomial (single-hump curve), the correlation between observed and modeled values was diminished. An analysis in the same form as shown in Figure 6, but using predicted values from a model with the third-order weightings, resulted in a lower regression r^2, as well as markedly larger residual values associated with observed values between 0.5 and 1.5. The third-order weighting model was less accurate than the nonweighted model.

Finally, it was assumed that the inoculation procedure for the infection bioassay adequately represents natural inoculation conditions. The upper range of inoculum potential from natural daily exposure in the field includes the level of inoculum potential applied in the bioassays (Figs. 1B and 2). It is possible that inoculum collecting on the plant surface over a full day would behave differently than inoculum applied at one time (1700 h) as in the bioassay. However, no assumption was made regarding the relative vigor or quantity of field inoculum (Fig. 2) compared with applied inoculum; rather, the level of infection produced by the different inocula (field or applied) under standard conditions (i.e., the inoculum potential) was the measure of similarity.

Unlike infection models that assign rate or asymptote effects to wetness duration or temperature individually (2,4), the model presented here does not include separate component functions for temperature and wetness duration. Instead, it combines the two as a calculation of d-h accumulated during wetness events (DH_w) and, therefore, does not attribute the upper infection limit to either temperature or wetness duration, but incorporates it into the saturation equation of the DH_w-dependent model. As noted previously, the assumption here is that differing final infection levels result from differing thermal-time durations available for the process. Alternatively, it is possible that the upper limit itself is affected by temperature, such that a lower proportion of the spores can succeed under marginally low temperatures than under more favorable temperatures, even with unlimited duration. In practice, the dark requirement for stem rust urediniospore germination would put an upper bound on duration (night length), so that the lowered upper limit due to low temperature may never be encountered when germination rates are slowed by low temperature.

The cumulative d-h format of the model permits the use of data from a variable environment, by calculating the DH_w in each short time interval and accumulating them over the course of several hours. Calculations therefore can be performed readily from the data collected at 15- or 30-min intervals by most automatic dataloggers, although one difficulty is the need to calculate or include sunset and sunrise times in the algorithm. This model, based only

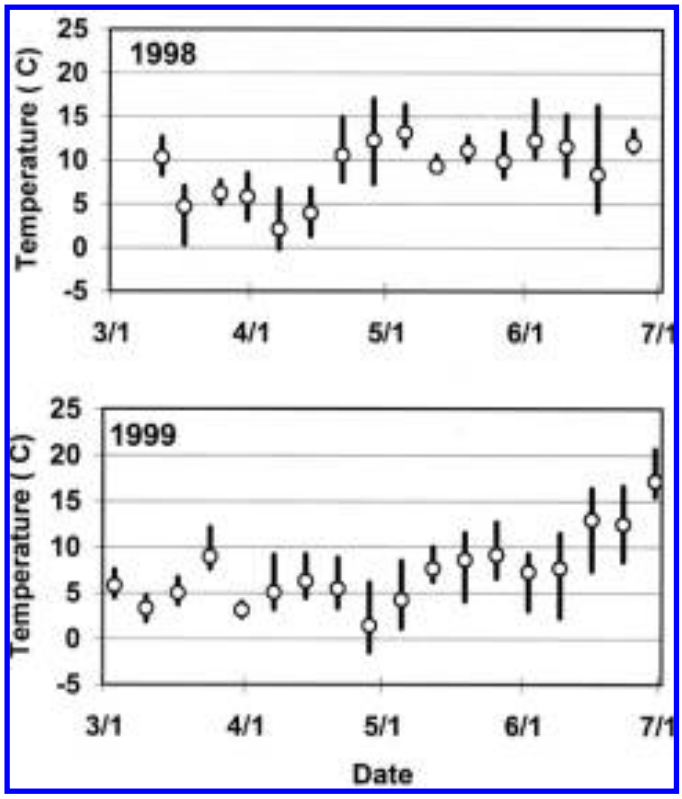

Fig. 7. Daily overnight temperature ranges (minimum and maximum) observed at biweekly intervals during March and June 1998 and 1999, in the Willamette Valley, OR. Temperature data are from sunset to 2 h past sunrise the following morning. Circles indicate average temperature during the period.

on temperature during periods of moisture, was able to account for at least 80% of the variance in our data (model development data as well as independent validation data) over three orders of magnitude in the value of log(infection level). The remaining 20% might be attributable to other environmental factors, but also may be a reflection of the inherent large variability common in field studies.

The model estimates the proportion of maximum possible infection, rather than an absolute level of infection. The ultimate use for this type of infection model, therefore, would be in conjunction with another submodel to estimate inoculum level. In the 2001 validation test data, three levels of inoculum over a 25-fold range were used, and the model-derived proportion was multiplied by each of the three maxima, which had been estimated from inoculations under standard conditions. The maximum for the highest inoculum level was $10^{3.4}$ pustules per 0.1 m^2, which approaches the highest inoculum level commonly seen in the field (Figs. 1B and 2). Over the 25-fold inoculum range, infection was proportional to inoculum level, and the resulting predictions were acceptable (Fig. 5B). At even higher inoculum levels, the saturation effect of increasing inoculum on infection level would have to be accounted for.

The model describes certain weather conditions as being nonconducive for infection, and provides a quantitative estimate for severity if infection conditions are conducive. In this way, it provides more information about the expected level of infection than a binary (infection/no infection) predictor, but there is a level of uncertainty or variance around the expected infection level. Whether that uncertainty is great enough to interfere with practical use of the model depends not only on the level of uncertainty but also on the way the model would be used. The intent is to use this infection model as a component of a more inclusive, daily time-step simulation model for rust increase; the inclusive model would include components for infection favorability (this article), amount and dispersal of inoculum, host susceptibility, and latent period (12). As a decision aid, this type of model will not necessarily have a single-event criterion for spray recommendation, but will depend on cumulative epidemic development. Epidemic prediction is most likely to be seriously affected by incorrect infection predictions if there is either substantial infection when none is predicted, or no infection when much is predicted. In the 50 trials of the validation tests (Fig. 7), there was only one case in which as many as $10^{1.2}$ infections per 0.1 m^2 (equivalent to approximately 1 pustule per mature tiller) were observed when the modeled level was near zero. This degree of infection could be important for disease management decisions if accumulated over many repeated occurrences, but not as a single event. Over-prediction by the model likewise does not appear serious, there being no cases of modeled severe infection where very low or no infection was observed. This level of uncertainty should not be a problem with practical use of the model, and an estimate of the variance may even be useful in developing a stochastic model for stem rust epidemics.

In developing a weather-based infection model, it is important to acknowledge the imperfect correspondence between environmental measurements and actual conditions where the plant and fungus interact. The measured canopy-level air temperature may differ from leaf surface temperature, particularly after sunrise. Onset and dry-off of leaf wetness is patchy within the canopy, and imperfectly represented by the presence of moisture on resistance-type leaf wetness sensors. Whatever the relationship between measured weather factors and the fundamental biology on the leaf surface, the requirement for successful epidemic modeling is that there be a reliable and accurate empirical prediction of infection from simple measurements as they are recorded by the monitoring equipment. The described approach appears to meet this requirement.

This model was developed for a single cultivar of perennial ryegrass. Other cultivars may show a different response, and further research with additional host genotypes is needed. However, it is likely that most of the difference could be accommodated by adjusting the value of maximum possible infection, rather than requiring a change in the relationship between weather and infection favorability. Likewise, the amount and vigor of inoculum (inoculum potential) and its availability (dispersal) could be accommodated in the term for maximum possible infection. Ultimately, the infection model will be combined with these maximum-infection estimates and a model for latent period duration (12), then adjusted for fungicide effects, to construct an epidemic model useful for stem rust management in seed crops of perennial ryegrass.

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